

SEQUENCE LISTING

<110> ENEA-Ente per le Nuove Tecnologie e l'Ambiente
Consiglio Nazionale delle Ricerche

<120> Method for the preparation of transgenic plants characterised by
Geminivirus lasting resistance

<130> PCT25622

<140> RM2003A000242
<141> 2003-05-19

<150> RM2003A000242
<151> 2003-05-19

<160> 12

<170> PatentIn version 3.2

<210> 1
<211> 630
<212> DNA
<213> Geminivirus TYLCSV

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aaattattca tcaaaatttg cagagaacta catgaaaatg gggaacctca tctccatatt 180
ctcatccaat tcgaaggaaa atacaattgt accaatcaac gattcttcga cctgggtatcc 240
ccaaccaggt cagcacattt ccatccgaac attcagggag ctaaatacgag ctccgacgtc 300
aagtcctata tcgacaagga cggagatggt cttgaatggg gtactttcca gatcgacgga 360
cgatctgcta ggggaggaca acagacagcc aacgacgctt acgcaaaggc aattaacgca 420
ggaagtaagt cgcaggctct tgatgtaatt aaagaattag cgcctagaga ttacgttcta 480
cattttcata atataaatag taatttagat aaggttttcc aggtgcctcc ggcaccttat 540
gtttctcctt ttttatcttc ttctttcgat caagttcctg atgaacttga acactggggt 600
tccgagaacg tcatggatgc cgctgcgcgg 630

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<223> TYLCSV Rep-210 modified sequence

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 35 40 45
 Glu Leu His Glu Asn Gly Glu Pro His Leu His Ile Leu Ile Gln Phe
 50 55 60
 Glu Gly Lys Tyr Asn Cys Thr Asn Gln Arg Phe Phe Asp Leu Val Ser
 65 70 75 80
 Pro Thr Arg Ser Ala His Phe His Pro Asn Ile Gln Gly Ala Lys Ser
 85 90 95
 Ser Ser Asp Val Lys Ser Tyr Ile Asp Lys Asp Gly Asp Val Leu Glu
 100 105 110
 Trp Gly Thr Phe Gln Ile Asp Gly Arg Ser Ala Arg Gly Gly Gln Gln
 115 120 125
 Thr Ala Asn Asp Ala Tyr Ala Lys Ala Ile Asn Ala Gly Ser Lys Ser
 130 135 140
 Gln Ala Leu Asp Val Ile Lys Glu Leu Ala Pro Arg Asp Tyr Val Leu
 145 150 155 160
 His Phe His Asn Ile Asn Ser Asn Leu Asp Lys Val Phe Gln Val Pro
 165 170 175
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 Pro Asp Glu Leu Glu His Trp Val Ser Glu Asn Val Met Asp Ala Ala
 195 200 205
 Ala Arg
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<220>
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 1 5 10 15

48

aca tac ccc aag tgt gac tta act aag gag aat gca ttg tcc cag ata 96
 Thr Tyr Pro Lys Cys Asp Leu Thr Lys Glu Asn Ala Leu Ser Gln Ile
 20 25 30
 act aac ttg caa act ccc act aac aag ttg ttc att aag att tgt agg 144
 Thr Asn Leu Gln Thr Pro Thr Asn Lys Leu Phe Ile Lys Ile Cys Arg
 35 40 45
 gaa ctt cac gag aat gga gaa cca cat ctt cat atc ttg ata cag ttc 192
 Glu Leu His Glu Asn Gly Glu Pro His Leu His Ile Leu Ile Gln Phe
 50 55 60
 gaa ggc aag tat aac tgc acc aac caa cgt ttc ttt gac ctt gtg tcc 240
 Glu Gly Lys Tyr Asn Cys Thr Asn Gln Arg Phe Phe Asp Leu Val Ser
 65 70 75 80
 cct acc aga tca gcc cat ttt cat cca aac atc cag ggt gct aag tcg 288
 Pro Thr Arg Ser Ala His Phe His Pro Asn Ile Gln Gly Ala Lys Ser
 85 90 95
 agt tca gac gtg aag tca tac att gac aaa gac ggg gat gtg ctc gag 336
 Ser Ser Asp Val Lys Ser Tyr Ile Asp Lys Asp Gly Asp Val Leu Glu
 100 105 110
 tgg gga act ttt cag ata gac ggt cga tcg gct aga gga ggt cag caa 384
 Trp Gly Thr Phe Gln Ile Asp Gly Arg Ser Ala Arg Gly Gly Gln Gln
 115 120 125
 aca gca aac gat gca tac gct aag gct atc aac gct gga tcc aag tca 432
 Thr Ala Asn Asp Ala Tyr Ala Lys Ala Ile Asn Ala Gly Ser Lys Ser
 130 135 140
 cag gca ctt gac gta atc aaa gag tta gct cct agg gat tat gtt ctt 480
 Gln Ala Leu Asp Val Ile Lys Glu Leu Ala Pro Arg Asp Tyr Val Leu
 145 150 155 160
 cat ttc cat aac atc aac agc aat ttg gac aaa gtg ttc caa gtg cca 528
 His Phe His Asn Ile Asn Ser Asn Leu Asp Lys Val Phe Gln Val Pro
 165 170 175
 ccg gct cct tac gtt tca cct ttc tta agt tct tca ttt gat cag gtt 576
 Pro Ala Pro Tyr Val Ser Pro Phe Leu Ser Ser Ser Phe Asp Gln Val
 180 185 190
 cca gat gag ctt gag cat tgg gtg tct gaa aac gtt atg gac gcc gca 624
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 195 200 205
 gcc cgt 630
 Ala Arg
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 <213> Artificial

<220>
 <223> Synthetic Construct

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25

30

Thr Asn Leu Gln Thr Pro Thr Asn Lys Leu Phe Ile Lys Ile Cys Arg
 35 40 45

Glu Leu His Glu Asn Gly Glu Pro His Leu His Ile Leu Ile Gln Phe
 50 55 60

Glu Gly Lys Tyr Asn Cys Thr Asn Gln Arg Phe Phe Asp Leu Val Ser
 65 70 75 80

Pro Thr Arg Ser Ala His Phe His Pro Asn Ile Gln Gly Ala Lys Ser
 85 90 95

Ser Ser Asp Val Lys Ser Tyr Ile Asp Lys Asp Gly Asp Val Leu Glu
 100 105 110

Trp Gly Thr Phe Gln Ile Asp Gly Arg Ser Ala Arg Gly Gly Gln Gln
 115 120 125

Thr Ala Asn Asp Ala Tyr Ala Lys Ala Ile Asn Ala Gly Ser Lys Ser
 130 135 140

Gln Ala Leu Asp Val Ile Lys Glu Leu Ala Pro Arg Asp Tyr Val Leu
 145 150 155 160

His Phe His Asn Ile Asn Ser Asn Leu Asp Lys Val Phe Gln Val Pro
 165 170 175

Pro Ala Pro Tyr Val Ser Pro Phe Leu Ser Ser Ser Phe Asp Gln Val
 180 185 190

Pro Asp Glu Leu Glu His Trp Val Ser Glu Asn Val Met Asp Ala Ala
 195 200 205

Ala Arg
 210

<210> 6
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<220>
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 1 5 10 15

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gtg cgt agg aga ctt aac ttt gac tct ccg tac acc tct cgt gca gct Val Arg Arg Arg Leu Asn Phe Asp Ser Pro Tyr Thr Ser Arg Ala Ala	96
gct ccc aca gtc cag ggc att aag agg cga tct tgg aca tac aga cct Ala Pro Thr Val Gln Gly Ile Lys Arg Arg Ser Trp Thr Tyr Arg Pro	144
atg tac agg aaa ccg agg atg tat agg atg tat cgt agc cca gat gtg Met Tyr Arg Lys Pro Arg Met Tyr Arg Met Tyr Arg Ser Pro Asp Val	192
cct cct ggt tgc gaa gga ccc tgc aag gtg caa tcg tat gag caa cgt Pro Pro Gly Cys Glu Gly Pro Cys Lys Val Gln Ser Tyr Glu Gln Arg	240
gac gat gtg aag cac acc gga gtt gtt cgt tgc gtt tct gat gtg act Asp Asp Val Lys His Thr Gly Val Val Arg Cys Val Ser Asp Val Thr	288
aga ggt tca ggt atc act cac agg gtg gga aag cgt ttc tgt att aag Arg Gly Ser Gly Ile Thr His Arg Val Gly Lys Arg Phe Cys Ile Lys	336
tct att tac ata ttg ggt aag atc tgg atg gac gag aat atc aag aaa Ser Ile Tyr Ile Leu Gly Lys Ile Trp Met Asp Glu Asn Ile Lys Lys	384
cag aat cac act aat cag gtt atg ttc ttt ctt gtg cga gat cga aga Gln Asn His Thr Asn Gln Val Met Phe Phe Leu Val Arg Asp Arg Arg	432
cca tac gga acc agc cca atg gac ttc ggc cag gtg ttt aat atg ttc Pro Tyr Gly Thr Ser Pro Met Asp Phe Gly Gln Val Phe Asn Met Phe	480
gat aac gag cca tct act gca act gtg aaa aat gat ttg cgt gat aga Asp Asn Glu Pro Ser Thr Ala Thr Val Lys Asn Asp Leu Arg Asp Arg	528
tat cag gtg atg aga aag ttc cat gca acg gtg gtt ggt ggt cct tct Tyr Gln Val Met Arg Lys Phe His Ala Thr Val Val Gly Gly Pro Ser	576
gga atg aaa gag caa tgt ctt ctg aaa aga ttc ttt aag atc aac act Gly Met Lys Glu Gln Cys Leu Leu Lys Arg Phe Phe Lys Ile Asn Thr	624
cat gtc gtc tat aac cac cag gag caa gcg aaa tat gag aat cac act His Val Val Tyr Asn His Gln Glu Gln Ala Lys Tyr Glu Asn His Thr	672
gaa aat gct ttg ttg tta tac atg gcc tgt acc cac gca tct aat cca Glu Asn Ala Leu Leu Leu Tyr Met Ala Cys Thr His Ala Ser Asn Pro	720
gtt tac gca acg ctt aag atc cgt atc tat ttc tat gac gct gtg aca Val Tyr Ala Thr Leu Lys Ile Arg Ile Tyr Phe Tyr Asp Ala Val Thr	768
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Ala Pro Thr Val Gln Gly Ile Lys Arg Arg Ser Trp Thr Tyr Arg Pro
 35 40 45

Met Tyr Arg Lys Pro Arg Met Tyr Arg Met Tyr Arg Ser Pro Asp Val
 50 55 60

Pro Pro Gly Cys Glu Gly Pro Cys Lys Val Gln Ser Tyr Glu Gln Arg
 65 70 75 80

Asp Asp Val Lys His Thr Gly Val Val Arg Cys Val Ser Asp Val Thr
 85 90 95

Arg Gly Ser Gly Ile Thr His Arg Val Gly Lys Arg Phe Cys Ile Lys
 100 105 110

Ser Ile Tyr Ile Leu Gly Lys Ile Trp Met Asp Glu Asn Ile Lys Lys
 115 120 125

Gln Asn His Thr Asn Gln Val Met Phe Phe Leu Val Arg Asp Arg Arg
 130 135 140

Pro Tyr Gly Thr Ser Pro Met Asp Phe Gly Gln Val Phe Asn Met Phe
 145 150 155 160

Asp Asn Glu Pro Ser Thr Ala Thr Val Lys Asn Asp Leu Arg Asp Arg
 165 170 175

Tyr Gln Val Met Arg Lys Phe His Ala Thr Val Val Gly Gly Pro Ser
 180 185 190

Gly Met Lys Glu Gln Cys Leu Leu Lys Arg Phe Phe Lys Ile Asn Thr
 195 200 205

His Val Val Tyr Asn His Gln Glu Gln Ala Lys Tyr Glu Asn His Thr
 210 215 220

Glu Asn Ala Leu Leu Leu Tyr Met Ala Cys Thr His Ala Ser Asn Pro
 225 230 235 240

Val Tyr Ala Thr Leu Lys Ile Arg Ile Tyr Phe Tyr Asp Ala Val Thr
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Asn

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 <222> (51)..(443)

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 <222> (231)..(231)
 <223> Point mutation from C (Rep-210 wild-type) to T

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 <221> misc_feature
 <222> (233)..(233)
 <223> Point mutation from C (Rep 210 wild-type) to G

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 Arg Ser Gly Arg Phe Ser Ile Lys Ala Lys Asn Tyr Phe Leu Thr Tyr
 5 10 15
 ccc aaa tgt gat tta aca aaa gaa aat gca ctt tcc caa ata aca aac 152
 Pro Lys Cys Asp Leu Thr Lys Glu Asn Ala Leu Ser Gln Ile Thr Asn
 20 25 30
 cta caa aca ccc aca aac aaa tta ttc atc aaa att tgc aga gaa cta 200
 Leu Gln Thr Pro Thr Asn Lys Leu Phe Ile Lys Ile Cys Arg Glu Leu
 35 40 45 50
 cat gaa aat ggg gaa cct cat ctc cat att ttg atc caa ttc gaa gga 248
 His Glu Asn Gly Glu Pro His Leu His Ile Leu Ile Gln Phe Glu Gly
 55 60 65
 aaa tac aat tgt acc aat caa cga ttc ttc gac ctg gta tcc cca acc 296
 Lys Tyr Asn Cys Thr Asn Gln Arg Phe Phe Asp Leu Val Ser Pro Thr
 70 75 80
 agg tca gca cat ttc cat ccg aac att cag gga gct aaa tcg agc tcc 344
 Arg Ser Ala His Phe His Pro Asn Ile Gln Gly Ala Lys Ser Ser Ser
 85 90 95
 gac gtc aag tcc tat atc gac aag gac gga gat gtt ctt gaa tgg ggt 392
 Asp Val Lys Ser Tyr Ile Asp Lys Asp Gly Asp Val Leu Glu Trp Gly
 100 105 110
 act ttc cag atc gac gga cga tct gct agg gga gga caa cag aca gcc 440
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447

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 <220>
 <223> Synthetic Construct

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Thr Asn Leu Gln Thr Pro Thr Asn Lys Leu Phe Ile Lys Ile Cys Arg
 35 40 45

Glu Leu His Glu Asn Gly Glu Pro His Leu His Ile Leu Ile Gln Phe
 50 55 60

Glu Gly Lys Tyr Asn Cys Thr Asn Gln Arg Phe Phe Asp Leu Val Ser
 65 70 75 80

Pro Thr Arg Ser Ala His Phe His Pro Asn Ile Gln Gly Ala Lys Ser
 85 90 95

Ser Ser Asp Val Lys Ser Tyr Ile Asp Lys Asp Gly Asp Val Leu Glu
 100 105 110

Trp Gly Thr Phe Gln Ile Asp Gly Arg Ser Ala Arg Gly Gly Gln Gln
 115 120 125

Thr Ala
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<210> 10
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 <213> Artificial

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 <223> Primer

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 <221> misc_feature
 <222> (1)..(30)
 <223> Primer for PCR C4 mutagenesis

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<210> 11

<211> 30
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<220>
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 <222> (1)..(30)
 <223> Primer for PCR C4 mutagenesis

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 <213> Geminivirus TYLCSV

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 agccctgatg tacctccggg ttgtgaaggt ccctgtaaag tgcagtcgta cgagcagcgt 240
 gatgacgtca agcataccgg tgttgctgctg tgtgttagtg atgtaactag gggttctggt 300
 attactcata gagttggtaa acgtttttgt atcaagtcaa tttatatatt aggaaagatt 360
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 gaaaatcata ctgagaatgc cttgttattg tatatggctt gtactcatgc ttctaaccga 720
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